Reviewed by: Krystyna K. Locke, Toxicologist KKL 5/10/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Combined chronic toxicity/ oncogenicity (rat)

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 36710
Project No.: 8-0126

Test Material: AC 84,777; Purity: 98.1%

Synonyms: Avenge; Difenzoquat

Study Number(s): 1626

Sponsor: American Cyanamid Company

Testing Facility: Food and Drug Research Laboratories, Inc.

Title of Report: Chronic Oral Toxicity Study in Rats with AC 84,777

Author(s): Baily, D. E.; Gallo, M. A.; and Cox, G. E.

Report Issued: September 19, 1975

Conclusions:

Systemic NOEL = 500 ppm (25 mg/kg), males and females

Systemic LEL = 2500 ppm* (125 mg/kg; decreased body weight gains in both sexes)

Oncogenic NOEL = > 5000 ppm (250 mg/kg; HDT, males and females)

Core classification: Minimum as a chronic feeding study
Minimum as an oncogenic study

*The high-dose group was fed diets containing 2500 ppm of the test material for 30 weeks and 5000 ppm thereafter. No reason was given for the increase, but decreased body weight gains were already observed at the 2500 ppm level.

This study was originally reviewed by Toxicology Branch in 1976, but that review (Attachment I) is too superficial by current standards. Also, no reference was made to the oncogenic

part of this study. This study was, therefore, currently fully evaluated and core-classified.

Experimental Procedures:

Test Animals

FDRL, Wistar-derived rats (initial body weights: 50-51 g) were used. Source of animals was not specified.

Study Design

The dose levels used were 0, 100, 500 or 2500/5000 ppm in the diet. After the 30th week, the 2500 ppm level was increased to 5000 ppm; reason for an increase was not given. There were 100 rats per sex in the control group and 60 per sex in each of the treated groups. Animals were assigned to groups randomly and were housed singly. Interim sacrifice took place at 90 days and included 10 animals/sex/group. Duration of the study was 104 weeks.

Diet

Purina Laboratory Chow and water were fed ad libitum. Preparation and storage of the experimental diets, and analyses for stability and concentration of the test compound were not specified.

Observation

Animals were observed daily for toxic signs and mortality.

Body Weight and Food Intake

These were recorded weekly for the first 12 weeks and monthly thereafter. An analysis of variance was conducted on body weight data for the 4, 13, and 26 week periods. Efficiency of food utilization (EFU) was also calculated for the first 12 test weeks.

Ophthalmological Examination

This examination was performed on all rats initially, at 3 months prior to interim sacrifice, and at the termination of the study.

Clinical Chemistry

These tests (see Attachment I) were performed on 6 rats/sex/group at 3, 6, 12, and 24 months. Hematology and urinalysis were also performed at 18 months.

Gross Pathological Examination

All animals on the study were examined.

Microscopic Examination

All tissues (25 or more; see Attachment I) were examined for all animals of the control and the high-dose groups sacrificed at 90 days and at 24 months, and also for all animals sacrificed moribund or found dead in these two groups. For the low-dose and mid-dose groups, at least 8 tissues per animal were examined for 10 animals per sex that were sacrificed at 90 days and 24 months (see Attachment I). Microscopic examinations also included all grossly abnormal sites from all animals, and tissues from all animals found dead or sacrificed moribund.

Organ Weights

At the interim and final sacrifices, organ weights and organ/body weight ratios were determined for all animals. (Organs that were weighed are listed in Attachment I).

Statistics

With the exception of body weights and food intake, none of the data were apparently statistically analyzed.

Results:

Toxic Signs

General appearance and behavior were comparable for the control and treated groups. Randomly occurring, palpable, subcutaneous mammary masses were observed in animals from all four groups.

Survival

Mortality was unaffected by the test material. Excluding the interim sacrifices, the mortality rate in the control, low-dose, mid-dose, and high-dose males was 69, 68, 60 and 56 percent, respectively. The corresponding rates for the female groups were 50, 50, 26, and 30 percent, respectively.

Body Weight Gains

Compared with the controls, body weight gains were slightly but consistently decreased in the high-dose males and females. These decreases ranged from 4 to 9 percent in the males and from 8 to 16 percent in the females. In the case of males, the decreases were statistically significant ($p = 0.05$) for the test weeks 4, 13, and 26, the only time intervals when statistical

significance was calculated. In the case of females, the decreases were statistically significant only at the test week 26.

Food Consumption and Utilization (EFU)

With the exception of a slight decrease (4%) in the EFU (grams of weight gained/100 g of food eaten) in the high-dose group, the test material had no effect on both food consumption and EFU.

Parameters Unaffected by Treatment with AC 84,777

The test material had no effect on ophthalmoscopic findings, hematology, clinical chemistry, urinalysis, organ weights and organ/body weight ratios.

Gross Pathology

These data were reported mostly for individual animals. A comment was also made in the summary of data that less gross pathology was found in the high-dose test animals than in the controls. Dose-unrelated gross findings were observed mostly in the lungs (collapsed, abscessed, mottled, pale, puffy, firm and/or nodular); pituitary (enlarged and masses); adrenals (enlarged); liver (mottled); thyroid (enlarged); kidneys (granular, fluid-filled); and testes (discolored and atrophied).

Non-neoplastic Findings

Predominant but mostly dose-unrelated non-neoplastic changes were observed in the adrenals (hypervolemia, peliosis, vascular dilatation and cortical and medullary vacuolation); heart (fibrosis); kidneys (calcification, casts, epithelial hyperplasia, fibrosis, polyposis, and dilatation of the renal pelvis; liver (vacuolation); lungs (bronchitis, fibrosis, chronic interstitial inflammation, macrophage aggregates, pneumonitis, and peribronchial lymphoid hyperplasia; pancreas (fibrosis and hyperplasia of islet cells; pituitary (congestion, cysts, cystic spaces hyperplasia); spleen (hematopoiesis and pigment); ovaries (cysts, cystic follicles, and interstitial gland formation); uterus (dilatation); and testes (atrophy and fibrosis).

There was a dose-related increased incidence of nodular vacuolation of the adrenal cortex in the females. The percent incidence of this lesion in the control, low- , mid-, and high-dose female groups was 0, 11.1, 18.4 and 21.7, respectively. The corresponding values for the male groups were 18.6, 9.2,0 and 1.7 percent, respectively.

Hyperplasia of the parathyroid was another predominant

lesion. The numerical incidence of this lesion for the control, low-, mid-, and high-dose male groups was 10, 8, 6, and 14, respectively. The corresponding values for the female groups were 20, 5, 2, and 5, respectively. Because the numbers of tissues examined were not reported for parathyroids, the percent incidence cannot be calculated.

Neoplastic Findings

With the exception of tumors summarized below, most of the remaining tumors were single incidences observed in one or two groups, males or females.

Predominant Tumors Diagnosed Microscopically in Rat

AC 84,777 (ppm) Organ and Tumor	0	100	500	5000[1]	0	100	500	5000[1]
	Males				Females			
	Percent Incidence							
Adrenals								
Adenoma of cortex[2]	14.4	1.8	11.8	15.2	17.3	26.7	21.0	15.0
Lungs								
Sarcoma (reticulum cell)	21.6	14.5	25.5	24.1	19.2	11.1	7.9	10.3
Pituitary								
Adenoma (chromophobe)	28.7	21.2	33.3	7.4	17.2	26.1	63.1	23.6
Hyperplasia[3]	10.6	9.1	7.4	7.4	11.8	21.7	0.0	9.1
Thyroid								
Adenocarcinoma	4.1	2.9	6.3	10.2	2.1	4.0	11.8	3.6
Adenoma	3.1	2.9	3.1	1.7	1.0	0.0	0.0	1.8
Mammary								
Adenocarcinoma	-	-	-	-	3.1	3.8	11.5	0.0
Fibroadenoma	-	-	-	-	12.3	15.4	7.7	5.4
Ovary								
Adenoma	-	-	-	-	22.2	23.2	22.2	20.7
Uterus								
Polyps	-	-	-	-	8.2	7.3	12.1	6.7

[1] Animals in this group were fed diets containing 2500 ppm of the test material for 30 weeks and 5000 ppm thereafter.

[2] According to the authors of this study, the rather high incidence of cortical adenoma in these animals is largely a result of the choice of nomenclature, cortical adenoma being used to designate small foci of slightly enlarged vacuolated cortical cells if the vascular flow pattern and presence of peripheral compression give an appearance of nodularity.

[3] Both chromophobe adenoma and hyperplasia were listed under the heading Tumors and Proliferation (page 139 of the submission). A comment was also made in the Results and Discussion section of the report that, in this study, chromophobe adenoma of the anterior lobe was defined as hyperplasia causing peripheral compression.

With the exception of thyroid adenocarcinoma in the mid-dose and high-dose male rats, none of the other tumors appear treatment-related. The authors of this report regard an increased incidence of thyroid adenocarcinoma in the males as insignificant. According to Dr. Lynnard J. Slaughter, Consulting Pathologist, Toxicology Branch, Hazard Evaluation Division, the historical incidence of thyroid adenocarcinoma in the male and female Wistar-derived rats is about 19 percent. The incidence of thyroid adenocarcinoma observed in this study is, therefore, within normal limits. According to Dr. Slaughter, the following increased incidences of tumors, listed in the above table, are also within normal limits for this strain of rats: adrenal cortical adenoma in low-dose females; pituitary adenoma in mid-dose males and females; pituitary hyperplasia in low-dose females; and mammary adenocarcinoma and uterine polyps in mid-dose females.

Maximum Tolerated Dose (MTD)

Based on decreased body weight gains in the high-dose males and females, it appears that MTD was reached. Although decreases in body weight gains were small (4 to 9% in males and 8 to 16% in females, compared with the controls), they were statistically significant ($p = 0.05$) and persisted throughout the study.

Comments

Although this study is about 13 years old and not all of the parameters were examined according to the currently accepted standards (for example, only 6 rats/sex/group were used for the hematology and clinical chemistry analyses; blood electrolytes were not determined; and quality assurance inspections were not performed), enough of the important and scientifically sound data are available to accept this study as a valid chronic feeding/oncogenic study.

Systemic NOEL = 500 ppm (25 mg/kg, males and females)

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Systemic LEL = 2500 ppm (125 mg/kg; decreased body weight gains in males and females)

Oncogenic NOEL = > 5000 ppm (250 mg/kg; HDT; males and females)

Core Classification: Minimum for each chronic feeding and oncogenic study.

Attachment I

2 Year Rat Feeding - Food and Drug Research Lab - 9/19/75

The material tested was identified as AC 84777 (Tech), Lot No. AC-1786-158; (98.1%). This material was fed to 60 rats of each sex per level of 100, 500, or 2500/5000 ppm. The 2500 ppm level was changed to 5000 ppm after the 30th week.

Observations and tests for effects included body weights, food consumption, opthalmoscopic examination, interim sacrice at day 90, and the following laboratory test:

hematocrit	glucose
hemoglobin	BUN
RBC	SGPT
WBC	SAP
differential count	urinalysis

Terminal studies included a histopathological examination of the following tissues from all rats of the control and high levels:

Brain	Large Intestine
Pituitary	Mesenteric Lymph Node
Eye	Urinary Bladder
Thyroids	Mammary Gland
Heart	Testes/Epididymis
Lung	Prostate
Liver	Ovary/Uterus
Spleen	Bone Marrow
Kidneys	Spinal Cord
Adrenals	Skeletal Muscle
Stomach	Rib Junction
Pancreas	Sciatic Nerve
Aorta	Tissue Masses
Small Intestine	All Lesions

The following tissues were also examined from 10 rats of each sex from each level:

lung	adrenals
heart	testes/epididymis
kidneys	ovary
liver	uterus
mammary	lesions

Terminal studies also included organ weights of the following organs from all animals:

Thyroids	adrenals
Heart	Testes/epididymis
Liver	Ovary
Spleen	Uterus
Kidneys	

Results: 2500/5000 ppm level - body weight gain was depressed for both sexes at the 52, 78 and 104 week periods.

500 ppm level - normal biological variations.

100 ppm level - normal biological variations.

Conclusion: The no effect has to be established at 500 ppm for this study due to the depressed body weight gain at the high level. This effect is not considered to be of a severe nature but rather a consistance one during a significant part of the study.

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Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

KKL 5/10/88
Budd 5/23/88

DATA EVALUATION REPORT

Study Type: Oncogenicity (mouse)

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 37923
Project No.: 8-0126

Test Material: AC 84777; purity not stated

Synonyms: Difenzoquat, Avenge

Study Number(s): 7310M

Sponsor: American Cyanamid Company.

Testing Facility: Pharmacopathic Research Laboratories, Inc. Laurel, MD

Title of Report: Eighteen Month Carcinogenicity Study on AC 84777

Author(s): Tegeris, A. S. and Underwood, P. C.

Report Issued: February 26, 1975

Conclusions:

Oncogenic NOEL : Could not be determined because too few animals (tissues) were examined microscopically at all dose levels and especially at the 2500 ppm level (HDT).

Classification: Core-Supplementary (Too few parameters were examined).

This study was originally reviewed by Toxicology Branch (TB) in 1976, but the one-page review is too superficial (see Attachment I), implies that this study is an acceptable oncogenic study (which is incorrect) and also contains errors.

Briefly, this study was performed as follows:

1. CD-1 mice, 60 of each sex per dose level (and not 120 as stated in TB review) were fed diets (Charles River 19RF Meal) containing 0 (control 1), 0 (control 2), 100, 500, or 2500 ppm of

the test material. No reason was given for using two control groups.

2. The animals were housed 10/sex/ cage; nothing was said about the identification of animals.

3. At 6 and 12 test months, 5 males and 5 females from each group were sacrificed (interim sacrifices).

4. The following parameters were examined:
   a. Body weights -- were recorded initially and every 2 weeks thereafter for the first three months;
   b. Mortality rate and causes of death;
   c. Gross necropsy -- was performed on all animals in the study; and
   d. Histopathology. According to the submitted report, full microscopic examination was performed on the following animals:
      -All animals sacrificed at 6 and 12 test months;
      -All animals with tumors or gross lesions detected at necropsy; and
      -Selected (how?) animals at the termination of the study (10 males and 10 females from each of the control groups, and from the high-dose group).

The following parameters were not examined:

1. Palpation for masses;
2. Body weights during the entire study;
3. Food consumption;
4. Differential blood count;
5. Organ weights;
6. Microscopic examination of tissues from all animals in the control and high-dose groups;
7. Lung, liver and kidneys were not examined in all animals; and
8. Data were not analyzed statistically.

Reported results:

1. Regarding cageside observations, fighting was reported but no mention was made of any toxic signs.

2. At the termination of the study, the survival rates (corrected for interim sacrifices) were 64, 62, 36, 78, and 50 percent for the 0, 0, 100, 500, and 2500 ppm male groups, respectively. The corresponding survival rates for the female groups were 68, 68, 72, 74, and 74 percent, respectively.

3. At the end of the first three months, high-dose males and females gained about 20 percent and 14 percent, respectively, less weight than did the combined control groups for each sex.

4. The predominant causes of death in all groups were chronic renal disease, pulmonary edema and/or hemorrhage, respiratory disease, and leukemia. In many instances, causes of death could not be determined.

5. All of the animals were examined grossly. Based on the individual data, the following animals were examined microscopically:

	Number of animals examined			
Test group	Males		Females	
	Nonsurvivors	Sacrificed	Nonsurvivors	Sacrificed
Control 1	12	7	8	12
Control 2	13	8	10	8
100 ppm	15	5	6	12
500 ppm	10	8	6	11
2500 ppm	13	1	3	3

In most instances, only tumors observed at necropsy and other gross lesions were examined microscopically. In most instances, only 2 to 6 organs (tissues) per animal were examined. Full microscopic examination was performed only on one or two animals per group.

It should be noted that in Table 4 of the submission (a part of which was reproduced in TB review) neither the number of tissues nor the number of animals examined was reported.

Most of the male and female survivors that were examined microscopically were sacrificed on test days 557-561 (about 18 months) and were probably scheduled sacrifices. Other survivors, examined microscopically, were sacrificed as shown on the next page:

Test group	Number examined		Day sacrificed
	Males	Females	
Control 1	--	1	181[1]
500 ppm	1	3	181
Control 1	1	1	375[2]
Control 2	1	3	375
100 ppm	1	1	375
500 ppm	1	1	375
2500 ppm	1	1	375
Control 1	--	1	499[3]

Yet, it was stated in the submitted report that all of the animals (5/sex/group), killed at 6 and 12 test months, were examined microscopically.

6. Most of the pathology reports are dated February 22, 23, or 25, 1975, that is, one to four days before the date of the study report (February 26, 1975). Also, one pathology report (for a control male that died after 516 days) is dated February 28, 1975, or two days after the date of the study report.

Conclusions:

The authors of this study conclude that: "The incidence of tumors in the mice exposed to various levels of AC 84777 for eighteen months is so low it makes it quite obvious that AC 84777, under the conditions of this experiment, is not carcinogenic to mice." Actually, too few animals (and tissues) were examined microscopically to conclude if AC 84777 is oncogenic. Also, too few parameters were examined (and none evaluated statistically) to determine whether or not a MTD was reached. This study can, therefore, be accepted only as Supplementary data.

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[1] About 6 months.

[2] About 12 months.

[3] About 16 months.

Attachment I

18 Month Mice Carcinogenic - Pharmacopathics Research Lab - 2/26/75

The material tested was identified as AC 84777. This material was fed to 120 CD-1 outbred albino mice of each sex per level of 0, 100, 500 and 2500 ppm.

Observations and tests for effects included body weights, interim sacrifice at six months and twelve months and mortality.

Terminal studies included a histopathological examination of all tumors and a complete examination of tissues from ten mice of each sex of the high dose group and control group.

Results: As is evident from the following chart, no carcinogenic activity was detected in this study:

Diagnosis	Male				Female			
	0 PPM	100 PPM	500 PPM	2500 PPM	0 PPM	100 PPM	500 PPM	2500 PPM
VASCULITIS	3							
TUMORS, BENIGN								
ADENOMA								
PULMONARY	6		1					
THYROID								1
CYSTADENOMA, OVARY							1	
FIBROMA	1							
HEMANGIOMA, CAVERNOUS, UTERINE							1	
LUTEOMA OF OVARY						1		
TUMORS, MALIGNANT								
CARCINOMA								
ADENOCARCINOMA								
HEPATOMA	3	1					1	
MAMMARY GLANDS						1	1	1
PANCREAS	1							

DIAGNOSIS	Male				Female			
	0 PPM	100 PPM	500 PPM	2500 PPM	0 PPM	100 PPM	500 PPM	2500 PPM
SARCOMA								
FIBROSARCOMA, UTERUS								1
LYMPHOSARCOMA, TESTIS			1					
RETICULUM CELL	1				1			
LEUKEMIA								
LYMPHOCYTIC, CHRONIC	4		2		6	2		
MYELOGENOUS, ACUTE					1			
THYMOMA			1					
METASTASIS								
LUNG					1			

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The other test parameters did not indicate a significant difference between the test and control findings.

The NEL = 2500 ppm